



**PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Arnon SHANI, et al

Serial No: 09/856,795

Group No. 1616

Filed: August 14, 2001

Examiner: Shaojia A. Jiang

For: SUSTAINED RELEASE POLYMER-BASED WATER INSOLUBLE BEADS

Attorney Docket No: U 013484-1

Commissioner for Patents

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DECLARATION UNDER 37 CFR 1.132

I, Professor Shlomo Magdassi, an Israeli citizen of 36 Hanerd St., Jerusalem 96626, Israel, declare as follows:

1. I am one of the co-inventors of the above application and am fully familiar with the invention described and claimed therein. I have personal, first-hand knowledge of the following experiments which we conducted to test the polymer-based emulsion formulations described in the application for release of volatile hydrophobic components, such as pheromones.
2. I have noted that in the Office Action issued in this case the examiner has stated the opinion that using gelatine instead of a conventional surfactant is not an inventive step, since the gelatine is known for its emulsification properties. However, it has now been discovered as described in the present application that the addition of gelatine serves two purposes, which are emulsification, and, more important, its ability to slow down the release of the pheromone, which is unexpected, if it functions only as a surfactant.
3. To test the effects of gelatine on the release rate of pheromones from calcium-alginate based microcapsules and to support the idea that besides being a simple surfactant in the prepared emulsions gelatine also acts as an inhibitor of the pheromone release the following formulations were prepared:

ID1: 2% Tween-80 (Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2 , without gelatine.

ID2: 0.25% gelatine (type B, 75 Bloom, Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2

ID3: 0.50% gelatine (type B, 75 Bloom, Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2

ID4: 0.75% gelatine (type B, 75 Bloom, Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2

ID5: 1% gelatine (type B, 75 Bloom, Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2

ID6: 2% gelatine (type B, 75 Bloom, Sigma), 1% alginate (Protanal LF10/60, FMC BioPolymer), 10% dodecyl acetate (Sigma), 30 min cross-linking with 0.25% CaCl_2

4. The gelatine layer surrounding the emulsion droplets is clearly visible under a polarized-light microscope (X400) as seen in Exhibit 1 attached hereto.
5. Laser confocal microscopy showed that the layer is indeed composed of gelatine. The gelatine layer may be distinguished from the pheromone by labelling the gelatine with FITC (fluorescein isothiocyanate) and dissolving Nile-Red in the pheromone. Attached hereto as Exhibit 2 are two Figures wherein the Figure on the left shows the laser confocal microscopy of the emulsion with both FITC ($\lambda_{\text{ex}} = 494 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$) and Nile-Red ($\lambda_{\text{ex}} = 540 \text{ nm}$, $\lambda_{\text{em}} = 630 \text{ nm}$) being irradiated while in the Figure on the right only FITC is irradiated. As shown in the photos, the gelatine is present in the whole field, meaning that it is not just adsorbed on the oil droplets, as do conventional surfactants.
6. Measurements of droplet size as a function of gelatine concentration confirmed that gelatine is capable to form the emulsion, at concentrations as low as 0.1%. While using 0.4% gelatine, emulsions having particles below 10 micrometers are obtained, which is suitable for the slow release application as seen in Exhibit 3 attached hereto.
7. In general, we found that the release rate of dodecyl acetate is directly related to the concentration of gelatine. The experimental results shown in Exhibit 4 attached hereto support this fact. The fastest release of dodecyl acetate is observed in a case of microcapsules prepared without gelatine where Tween-80 is used instead as another surfactant to stabilise the prepared emulsion. As the concentration of gelatine increases the release rate of dodecyl acetate decreases.
8. This experimental evidence that gelatine affects the release rate of dodecyl acetate was also supported by scanning electron micrographs of cross-sections of capsules with gelatine of different concentrations (and one without gelatine) as seen in Exhibit 5 attached hereto
9. The SEM micrographs of Exhibit 5 clearly show that as the concentration of gelatine increases the porosity decreases. Calculation of the average pore size in these matrices gives us the quantitative estimation of the effect. The

Figure of Exhibit 6 attached hereto shows that the average pore size of the matrices significantly decreases as gelatine concentration increases.

10. Based on these experimental results we came to conclusion that gelatine distinct from other surfactants like Tween-80 and Brij-78 widely used to stabilise various emulsions. The effect of gelatine on the release rate of active ingredient from microcapsules under consideration is unique in that besides being a conventional surfactant it can significantly inhibit the release of the active compound. Possible explanations to the fact that gelatine slows the release rate out of the matrices can be either one or both of the following:
- a. A physical barrier is formed by a multilayer of gelatine around each droplet of the active compound. The multilayer gets thicker as gelatine concentration increases.
 - b. Besides surrounding the droplets gelatine is also dispersed in the emulsion and forms another barrier that is thicker with higher concentration of gelatine so the average pore size of the matrices significantly decreases as gelatine concentration increases.
11. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity or the application of any patent issued thereon.

Date:

16 / 12 / 03



Professor Shlomo Magdassi